Synthesis and Conformation in the Solid State of Oligopeptides with the L-Alanyl-L-leucylglycyl Sequence in which Some Glycines are replaced with N-Methylglycines

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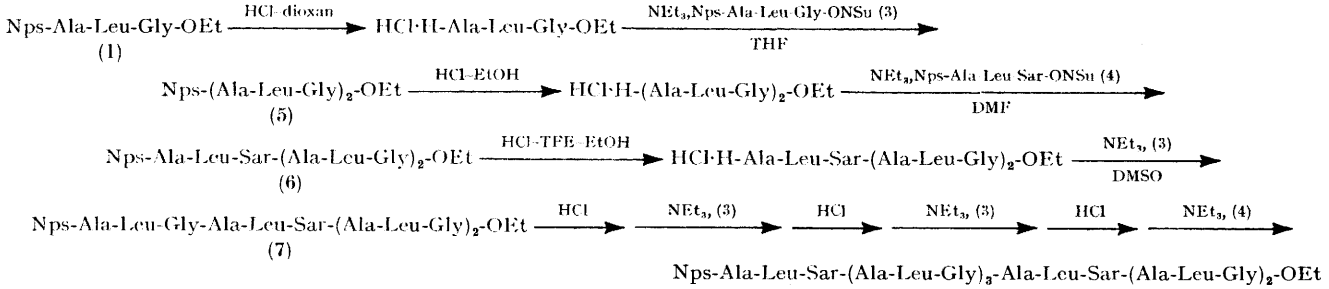
A number of oligopeptides consisting of L-alanyl-L-leucylglycyl sequences in which some glycyl residues are replaced with *N*-methylglycyl residues were prepared by the fragment condensation method using Nps-L-Ala-L-Leu-Gly-ONSu and Nps-L-Ala-L-Leu-Sar-ONSu. Conformations in the solid state of the peptides were examined by i.r. spectroscopy. The shortest peptide length for the formation of an α-helix is three successive L-alanyl-L-leucylglycyl residues situated between L-alanyl-L-leucylsarcosyl residues.

PEPTIDES potentially capable of forming α-helices can take the α-helical conformation if they have chain lengths longer than certain definite lengths known as critical peptide lengths.[1] At the critical peptide length, the α-helix is stabilized to a degree that just maintains the helical conformation. Small changes in stabilization, brought about by perturbations in the peptide system, should therefore affect the critical peptide length. We have thus embarked on a study of peptides containing some *N*-methylated amino-acids which, due to their inability to form hydrogen bonds, are a suitable source of perturbation. Comparison of the critical peptide lengths for the formation of the α-helix for peptides with and without the *N*-methylated amino-acids should provide direct evidence for the role of hydrogen bonding in the stabilization of the α-helix. We now report the synthesis and conformation in the solid state of peptides containing the L-alanyl-L-leucylglycyl sequence in which some glycyl residues are replaced by *N*-methylglycines (sarcosines).

Peptides were synthesized by the fragment-condensation method using the *N*-hydroxysuccinimide (ONSu) active ester of the tripeptide units. The tripeptide units Nps-L-Ala-L-Leu-Gly-OEt (1) and Nps-L-Ala-L-Leu-Sar-OEt (2) were prepared by the *o*-nitrophenylsulphenyl (Nps)–*N*-carboxy-amino-acid anhydride (NCA) method.[2] Treatment of glycine or sarcosine ethyl ester with the Nps-NCA of L-leucine was followed by deprotection of the Nps-group with hydrochloric acid and reaction with the Nps-NCA of L-alanine to give (1) or (2). These tripeptide esters were saponified to give the free acids, which were then esterified with HONSu and dicyclohexylcarbodi-imide to give the tripeptide active esters Nps-L-Ala-L-Leu-Gly-ONSu (3) and Nps-L-Ala-L-Leu-Sar-ONSu (4). All reactions were carried out in tetrahydrofuran (THF).

The syntheses of the oligopeptides were achieved from (1) as the starting peptide by its reaction with (3) or (4). The sarcosyl residue could be introduced into the appropriate position of the glycyl residue in the L-alanyl-L-leucylglycyl sequence during the elongation of the peptide chains using (4) instead of (3). A typical synthesis is that of the henicosapeptide Nps-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_3$-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_2$-OEt. (1) was treated with 2N-hydrochloric acid in dioxan to give the tripeptide ester hydrochloride, which was allowed to react with (3) in the presence of triethylamine in THF for 5 h at room temperature to give the hexapeptide Nps-(L-Ala-L-Leu-Gly)$_2$-OEt (5). The hexapeptide (5) was treated with 4N-hydrochloric acid in ethanol to give the hydrochloride, which was allowed to react with (4) in dimethylformamide (DMF) to yield the nonapeptide Nps-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_2$-OEt (6). This was dissolved in trifluoroethanol (TFE) and to the solution was added 4N-hydrochloric acid in ethanol to remove the Nps-group. The resulting nonapeptide ester hydrochloride was treated with (3) in dimethyl sulphoxide (DMSO) to give a dodecapeptide Nps-L-Ala-L-Leu-Gly-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_2$-OEt (7) which, on treatment by the same procedure as above followed by deprotection of the Nps-group with hydrochloric acid in TFE–ethanol and reaction with (3) or (4) in DMSO, gave finally the henicosapeptide. All reactions proceeded in clear solution in DMSO. The tripeptide active esters (3) or (4) were used in excess (1.5 mol equiv. with respect to the peptide



SCHEME

TABLE 1

Oligopeptides

Nps-peptide-OEt [a]	Yield (%)	M.p. (°C)	R_F [c]	$[\alpha]_D$	Found (%) C	H	N	Required (%) C	H	N
ALSALG	86	102—104	0.71	−43.5 [d]	53.4	7.0	14.3	53.5	7.1	14.3
ALGALSALG	89	155—157	0.33	−39.1 [d]	53.7	7.3	15.0	53.8	7.3	14.95
$(ALG)_2ALSALG$	80	102—104	0.13	−39.6 [d]	54.1	7.5	15.3	54.0	7.4	15.5
$(ALG)_3ALSALG$	91	239—241	0.14	−39.0 [e]	54.3	7.7	15.6	54.1	7.5	15.8
$(ALG)_4ALSALG$	93	266—268	0.18	−40.9 [f]	54.3	7.7	15.9	54.2	7.6	16.0
$ALS(ALG)_2$	90	195—197	0.13	−39.0 [d]	53.9	7.1	14.8	53.8	7.3	14.95
$ALGALS(ALG)_2$	82	116—118	0.36	−43.6 [d]	54.0	7.6	15.3	54.0	7.4	15.5
$(ALG)_2ALS(ALG)_2$	91	178—180	0.25	−41.6 [e]	54.0	7.7	15.6	54.1	7.5	15.8
$(ALG)_3ALS(ALG)_2$	94	213—218 [b]	0.06	−38.2 [e]	54.5	7.7	16.1	54.2	7.6	16.0
$(ALG)_4ALS(ALG)_2$	90	261—266 [b]	0.12	−44.3 [e]	54.4	7.5	16.1	54.3	7.6	16.2
$ALS(ALG)_3ALS(ALG)_2$	89	233—238 [b]	0.09	−51.6 [e]	54.4	7.8	15.9	54.5	7.7	16.1
$(ALS)_2(ALG)_2$	79	156—158	0.09	−46.5 [d]	54.6	7.5	15.2	54.5	7.7	15.3
$ALG(ALS)_2(ALG)_2$	83	136—138	0.13	−43.1 [d]	54.6	7.7	15.3	54.5	7.6	15.6
$(ALG)_2(ALS)_2(ALG)_2$	90	170—172	0.08	−46.9 [d]	54.6	7.7	15.9	54.5	7.6	15.9
$ALS(ALG)_4$	94	264—267 [b]	0.04	−25.6 [f]	54.3	7.7	16.0	54.1	7.5	15.8
$ALGALS(ALG)_4$	90	265—270	0.07	−29.1 [f]	54.3	7.7	16.2	54.2	7.6	16.0
$(ALS)_2(ALG)_4$	93	247—251 [b]	0.08	−42.6 [f]	54.7	7.7	15.6	54.5	7.6	15.9
$ALS(ALG)_2ALS(ALG)_2$	92	226—230 [b]	0.13	−44.4 [e]	54.3	7.8	15.7	54.5	7.6	15.9
$ALGALS(ALG)_2ALS(ALG)_2$	91	172—174	0.08	−43.6	54.3	7.6	16.2	54.5	7.7	16.1

[a] Abbreviations: A, L-alanyl; L, L-leucyl; G, glycyl; S, sarcosyl. [b] Decomposed. [c] T.l.c. on silica gel using HFIP–benzene (1 : 1 v/v) as eluant. [d] *c*, 0.5 in DMF. [e] *c*, 0.5 in DMSO. [f] *c*, 0.5 in TFE.

ester hydrochloride) to ensure complete reaction of the peptide ester. After the reaction, the excess of the active ester present in the product was easily removed by simple washing with organic solvents such as THF or methanol. The crude product was purified by recrystallization from DMF, DMSO, or TFE. All products obtained showed a single spot on t.l.c. The results of the peptides are listed in Table 1.

This synthesis of peptides contains two principal experimental processes : deprotection of the *N*-protecting group and reaction of the resulting amine component with the peptide active ester. In order to achieve successful results for both processes, it is very important to obtain a clear solution in which the reaction can proceed easily, since the solubility of peptides consisting of amino-acids having non-polar side chains, such as L-alanine and L-leucine in this study, becomes very low in the organic solvents used in these processes as the chain length increases. Choice of solvent system is therefore most important for a successful synthesis of these peptides. In this study, a variety of solvent systems was used in each reaction process as the peptide chain length increased. For removal of the Nps-group from the peptide derivatives by the action of hydrochloric acid, dioxan, ethanol, and TFE–ethanol were used as solvents for the peptides of shortest, short, and long chain lengths, respectively. On the other hand, the reaction of the resulting peptide ester hydrochloride with the active ester was carried out in THF, DMF, and DMSO for the peptides with shortest, long, and longest chain lengths, respectively. Fortunately, the peptides containing sarcosines have a rather higher solubility than those without and the reaction proceeded in a clear solution to give a pure product in high yield after simple purification by recrystallization.

Conformational characterization of these peptides was achieved by i.r. spectroscopy. I.r. bands characteristic of a α-helix and a β-structure for peptides having the L-alanyl-L-leucylglycyl sequence are known: bands at **1 657**, **1 545**, **527**, **466**, **393**, and **364** cm^{-1} for the α-helix, and bands at **1 700**, **1 636**, and **441** cm^{-1} for the β-structure.[3] We thus characterized the conformation of our peptides by their i.r. bands. The results of the characterization are summarized in Table 2.

The results reveal many interesting facts. First, the presence of sarcosyl residues in the middle of the peptide chains prevents the formation of the α-helix. This was demonstrated by the formation of a β-structure in peptides (D), (G), (J), and (K), which have a peptide length above pentadecapeptide which is the critical peptide length for the formation of an α-helix of a peptide without sarcosyl residues.[1] However, the presence of a successive sequence of four L-alanyl-L-leucylglycyl residues at the C- or N-terminal positions permits

TABLE 2

Conformations of oligopeptides in the solid state

Peptide	Conformation
(A) Nps-Ala-Leu-Sar-$(Ala-Leu-Gly)_4$-OEt	α-helix
(B) Nps-Ala-Leu-Gly-Ala-Leu-Sar-$(Ala-Leu-Gly)_4$-OEt	α-helix
(C) Nps-$(Ala-Leu-Sar)_2$-$(Ala-Leu-Gly)_4$-OEt	α-helix
(D) Nps-$(Ala-Leu-Gly)_3$-Ala-Leu-Sar-Ala-Leu-Gly-OEt	β-structure
(E) Nps-$(Ala-Leu-Gly)_4$-Ala-Leu-Sar-Ala-Leu-Gly-OEt	α-helix
(F) Nps-Ala-Leu-Sar-$(Ala-Leu-Gly)_3$-Ala-Leu-Sar-Ala-Leu-Gly-OEt	α-helix
(G) Nps-$(Ala-Leu-Gly)_3$-Ala-Leu-Sar-$(Ala-Leu-Gly)_2$-OEt	β-structure
(H) Nps-$(Ala-Leu-Gly)_4$-Ala-Leu-Sar-$(Ala-Leu-Gly)_2$-OEt	α-helix
(I) Nps-Ala-Leu-Sar-$(Ala-Leu-Gly)_3$-Ala-Leu-Sar-$(Ala-Leu-Gly)_2$-OEt	α-helix
(J) Nps-$(Ala-Leu-Gly)_2$-Ala-Leu-Sar-$(Ala-Leu-Gly)_2$-OEt	β-structure
(K) Nps-Ala-Leu-Gly-Ala-Leu-Sar-$(Ala-Leu-Gly)_2$-Ala-Leu-Sar-$(Ala-Leu-Gly)_2$-OEt	β-structure

formation of the α-helix in the peptide chains longer than pentadecapeptides with sarcosyl residues. This was demonstrated by the existence of the α-helical conformation in peptides (A), (B), (C), (E), and (H). However, a successive sequence of three L-alanyl-L-leucylglycyl residues at the terminal positions does not provide a sufficient chain length to form the α-helix. This was shown by formation of a β-structure for peptides (D) and (G). An interesting fact is that a successive sequence of three L-alanyl-L-leucylglycyl residues permits formation of the α-helix when it is present in the middle of the peptide chain as demonstrated by peptides (F) and (I). A successive sequence of two L-alanyl-L-leucylglycyl residues is not enough to form the α-helix even if it is present in the middle of the peptide chains. This was demonstrated by formation of the β-structure in the peptide (K). From these results, we conclude that α-helix formation for peptides consisting of L-alanyl-L-leucylglycyl residues in which some of the glycyl residues are replaced with sarcosyl residues needs at least the presence of three L-alanyl-L-leucylglycyl residues joined by the L-alanyl-L-leucylsarcosyl residue in the middle of the peptide chains. This means that the shortest unit that can form an α-helix is the sequence Sar-(L-Ala-L-Leu-Gly)$_3$-L-Ala-L-Leu-Sar.

Apart from this shortest unit required for formation of the α-helix, we were interested in the formation of the α-helix of the peptide Nps-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_4$-OEt (A), since we can discuss this result in relation to the role of the peptide moieties at the terminal and internal positions in the peptide chains on the conformation of the α-helix. Peptide (A) forms an α-helix although it has a sarcosyl residue in the same peptide length as the critical length for the formation of the α-helix of the peptides lacking a sarcosyl residue. This means that the presence of a sarcosyl residue at the third position from the N-terminus does not interfere with α-helix formation and that the α-helix is formed with the middle moiety of the peptide chain. We have already reported in a conformational study of other peptide systems [4] that in the critical peptide chain length which permits α-helix formation, three-amino-acid residues at the terminal positions do not participate in the α-helix formation but serve as supports for the formation of this conformation built by the peptide moiety in the middle of the chains. The formation of an α-helix in the peptide (A) suggests that this concept of the role of the terminal peptide moieties is applicable to the moieties containing *N*-substituted amino-acids. Since the N-terminal L-alanyl-L-leucylsarcosyl residue serves only as the support for the α-helix formation, the presence of the sarcosyl residue at that position does not prevent the formation of this conformation. This result was further supported by α-helix formation for peptides (B) and (C), in which the N-terminal L-Ala-L-Leu-Gly-L-Ala-L-Leu-Sar and (L-Ala-L-Leu-Sar)$_2$ sequences are the support.

The failure of α-helix formation for peptides (D) and (G) is also interesting. These peptides have the N-terminal sequence Nps-(L-Ala-L-Leu-Gly)$_3$-L-Ala-L-Leu-Sar-. Since the N-terminal tripeptide L-Ala-L-Leu-Gly should be the support, the peptide moiety which can participate in the α-helix formation is -(L-Ala-L-Leu-Gly)$_2$-L-Ala-L-Leu-Sar-, which is not long enough for the α-helix formation shown by the peptide (K) which has the sequence L-Ala-L-Leu-Gly-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_2$-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_2$-.
Addition of a tripeptide moiety L-Ala-L-Leu-Gly or L-Ala-L-Leu-Sar, serving as the support to the peptides (D) and (G), permits α-helix formation, as was demonstrated by peptides (E), (F), (H), and (I), which have the internal peptide sequence -(L-Ala-L-Leu-Gly)$_3$-L-Ala-L-Leu-Sar-.

EXPERIMENTAL

Melting points were measured in capillary tubes with a Yamato MP-21 apparatus. Optical rotations were measured with a JASCO DIP-SL automatic polarimeter. I.r. spectra in the amide I and II regions were recorded for KBr discs with a JASCO A-1 spectrophotometer. Far-i.r. spectra were recorded for Nujol mulls with a JASCO IR-F spectrophotometer.

Nps-L-Ala-L-Leu-Gly-ONSu.—This compound was prepared by the method reported earlier.[5]

Nps-L-Leu-Sar-OEt.—To sarcosine ethyl ester hydrochloride (7.7 g, 0.05 mol) in chloroform (100 ml) was added triethylamine (7 ml) and Nps-L-leucine NCA (17.2 g, 0.055 mol), and the mixture was stirred for 2 h at room temperature. Evaporation under reduced pressure gave an oil, which was dissolved in ethyl acetate (300 ml). The solution was washed with 5% aqueous sodium hydrogencarbonate, water, 5% citric acid, and water, and dried (Na_2SO_4). Evaporation under reduced pressure gave an oil which was chromatographed on silica gel with chloroform–benzene (1 : 1 v/v) as eluant to give, after evaporation and drying of the main fraction *in vacuo*, the pure dipeptide derivative as a yellow oil main product (17.5 g, 82%); R_F 0.71 (ethyl acetate–benzene 1 : 1 v/v); $[\alpha]_D$ −54.7 (*c* 1.0 in methanol).

Nps-L-Ala-L-Leu-Sar-OEt.—To Nps-L-Leu-Sar-OEt (15.3 g, 0.04 mol) in 2N-hydrochloric acid in dioxan (40 ml) was added diethyl ether (400 ml), when a yellow oil precipitated. The precipitate was repeatedly extracted with diethyl ether until the yellow colour disappeared. The resulting oil was then allowed to stand in diethyl ether overnight in a refrigerator when crystallization occurred. Recrystallization from ethanol gave the pure dipeptide ester hydrochloride which was then dissolved in THF (100 ml), and triethylamine (5.6 ml) added. The resulting crystals were filtered off, and to the filtrate was added with stirring Nps-L-alanine NCA (11.9 g, 0.044 mol). Reaction was continued for 2 h at room temperature when the solution was diluted with ethyl acetate (300 ml), washed with $NaHCO_3$ and citric acid, and dried (Na_2SO_4). Evaporation gave an oil (15.7 g, 86%) which gave a single spot on t.l.c., R_F 0.17 (ethyl acetate–benzene, 1 : 1 v/v); $[\alpha]_D$ −75.4 (*c*, 0.2 in methanol).

Nps-L-Ala-L-Leu-Sar-OH.—To the above tripeptide ester (13.6 g, 0.03 mol) in acetone (50 ml) was added 1N-sodium hydroxide (30 ml). The solution was stirred for 1 h at room temperature, then diluted with water (100 ml), extracted with diethyl ether (50 ml), and the extract was

acidified to pH 3 with 10% citric acid. The solution was extracted with ethyl acetate (200 ml) and the extracts were washed with 10% aqueous sodium chloride and dried ($MgSO_4$). Evaporation under reduced pressure gave an oil which crystallized on addition of hexane. Recrystallization from ethyl acetate gave the pure *free acid* (12.0 g, 94%), m.p. 57—59 °C; R_F 0.44 (ethyl acetate–benzene, 1 : 1 v/v); $[\alpha]_D$ −67.1 (*c*, 0.2 in methanol) (Found: C, 50.5; H, 6.4; H, 13.5. $C_{18}H_{26}N_4O_6S$ requires C, 50.7; H, 6.15; N, 13.1%).

Nps-L-Ala-L-Leu-Sar-ONSu.—To the above free acid (10.7 g, 0.025 mol) in THF (100 ml) was added *N*-hydroxysuccinimide (5.75 g, 0.05 mol). The solution was cooled to −5 °C and dicyclohexylcarbodi-imide (6.12 g, 0.03 mol) was added with stirring. Stirring was continued at −5 °C for 5 h when the mixture was diluted with ethyl acetate (300 ml). The resulting crystals of the urea were filtered off, and the filtrate was washed with 5% $NaHCO_3$ and water, and dried ($MgSO_4$). Evaporation under reduced pressure and recrystallization from THF gave yellow *crystals* (11.9 g, 88%), m.p. 162—164 °C; R_F 0.88 (THF); $[\alpha]_D$ −34.4 (*c*, 0.2 in DMF) (Found: C, 49.1; H, 5.3; N, 13.8. $C_{22}H_{29}N_5O_8S$ requires C, 50.5; H, 5.6; N, 13.4%).

Typical Example of the Fragment Condensation. Nps-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_2$-OEt.—To Nps-(L-Ala-L-Leu-Gly)$_2$-OEt [5] (6.64 g, 0.01 mol) in THF (20 ml) was added 4N-hydrochloric acid in ethanol (5 ml). On addition of diethyl ether (200 ml) the hexapeptide ester hydrochloride crystallized out. The crystals were collected on a glass filter, washed with diethyl ether until the yellow colour disappeared, and recrystallized from ethanol to give the pure hydrochloride (5.5 g, 100%). The hydrochloride was dissolved in DMF (40 ml), and triethylamine (1.4 ml) was added followed by Nps-L-Ala-L-Leu-Sar-ONSu (8.1 g, 0.015 mol). The mixture was stirred at room temperature for 5 h and then diluted with water (300 ml) and extracted with ethyl acetate (2 × 200 ml). The combined extract was washed with 5% aqueous sodium hydrogencarbonate, water, 5% citric acid, and water, dried (Na_2SO_4), and evaporated under reduced pressure. Addition of hexane (200 ml) to the residue gave crystals which were collected on a glass filter, washed with diethyl ether, and recrystallized from warm THF.

Nps-(L-Ala-L-Leu-Gly)$_2$-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_2$-OEt.—To Nps-L-Ala-L-Leu-Gly-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_2$-OEt (1.16 g, 0.001 mol) in TFE (2 ml) was added 4N-hydrochloric acid in ethanol (0.5 ml). Addition of diethyl ether (100 ml) gave crystals which were collected on a glass filter, washed with diethyl ether, and recrystallized from TFE to give the pure hydrochloride, m.p. 167—169 °C; $[\alpha]_D$ −74.3° (c, 0.1 in methanol). The hydrochloride was dissolved in DMSO (20 ml), and triethylamine (0.14 ml) was added followed by Nps-L-Ala-L-Leu-Gly-ONSu (0.79 g, 0.001 5 mol), and stirring was continued for 5 h at room temperature. Addition of water (200 ml) gave crystals which were collected on a glass filter, washed with ethyl acetate and diethyl ether, and recrystallized from DMSO.

Nps-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_3$-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_2$-OEt.—To Nps-(L-Ala-L-Leu-Gly)$_3$-L-Ala-L-Leu-Sar-(L-Ala-L-Leu-Gly)$_2$-OEt (1.63 g, 0.001 mol) in TFE was added 4N-hydrochloric acid in ethanol (0.5 ml) followed by diethyl ether. The hydrochloride which crystallized out was washed with diethyl ether and recrystallized from TFE, m.p. 212—214 °C; $[\alpha]_D$ −81.0° (*c*, 0.1 in methanol). The hydrochloride was dissolved in DMSO (10 ml) and treated with triethylamine (0.14 ml) and Nps-L-Ala-L-Leu-Sar-ONSu (0.81 g, 0.001 5 mol). After stirring for 5 h at room temperature, the mixture was diluted with water (200 ml) to precipitate the product, which was collected on a glass filter, washed with methanol, THF, and diethyl ether, dried, and recrystallized from TFE.

Reprecipitation of the Peptides.—The peptide (500 mg) was dissolved in heptafluoroisopropyl alcohol (HFIP). Addition of diethyl ether (300 ml) precipitated the peptide which was collected on a glass filter, washed with diethyl ether, and dried.

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